

WHAT IS CLAIMED IS:

1. A method of diagnosis, the method comprising:

(a) providing a test sample of breast tissue;

(b) determining the level of expression in the test sample of a gene selected
5 from those listed in Table 1; and

(c) if the gene is expressed in the test sample at a lower level than in a control
normal breast tissue sample, diagnosing the test sample as containing cancer cells.

2. A method of determining the grade of a ductal carcinoma in situ (DCIS), the
10 method comprising:

(a) providing a test sample of DCIS tissue;

(b) deriving a test expression profile for the test sample by determining the
level of expression in the test sample of ten or more genes selected from those listed in
Tables 2-16;

(c) comparing the test expression profile to control expression profiles of the
15 ten or more genes in control samples of high grade, intermediate grade, and low grade DCIS;

(d) selecting the control expression profile that most closely resembles the test
expression profile; and

(e) assigning to the test sample a grade that matches the grade of the control
20 expression profile selected in step (d).

3. The method of claim 2, wherein the ten or more genes are 25 or more genes.

4. The method of claim 2, wherein the ten or more genes are 50 or more genes.

5. The method of claim 2, wherein the ten or more genes are 100 or more genes.

6. The method of claim 2, wherein the ten or more genes are 200 or more genes.

7. The method of claim 2, wherein the ten or more genes are 500 or more genes

8. A method of determining the likelihood of a breast cancer being DCIS or invasive breast cancer, the method comprising:

(a) providing a test sample of breast tissue;

(b) determining the level of expression in the test sample of a gene selected from the group consisting of a gene encoding CD74, a gene encoding MGC2328, a gene encoding S100A7, a gene encoding KRT19, a gene encoding trefoil factor 3 (TFF3), a gene encoding osteonectin, and a gene identified by a SAGE tag consisting of the nucleotide sequence CTGGGCGCCC;

(c) determining whether the level of expression of the selected gene in the test sample more closely resembles the level of expression of the selected gene in control cells of (i) DCIS or (ii) invasive breast cancer; and

(d) classifying the test sample as: (i) likely to be DCIS if the level of expression of the gene in the test sample more closely resembles the level of expression of the gene in DCIS cells; or (ii) likely to be invasive breast cancer if the level of expression of the gene in the test sample more closely resembles the level of expression of the gene in invasive breast cancer cells.

9. A method of predicting the prognosis of a breast cancer patient, the method comprising:

(a) providing a sample of primary invasive breast cancer tissue from a test patient; and

(b) determining the level of expression in the sample of a gene encoding S100A7 or a gene encoding fatty acid synthase (FASN),

wherein a level of expression higher than in a control sample of primary invasive breast carcinoma from a patient with a good prognosis is an indication that the prognosis of the test patient is poor.

10. A method of diagnosis comprising:

(a) providing a test sample of breast tissue comprising a test stromal cell; and

(b) determining the level of expression in the stromal cell of a gene selected from those listed in Tables 7, 8, 10, 15, and 16, wherein the gene is one that is expressed in a

cell of the same type as the test stromal cell at a substantially higher level when present in breast cancer tissue than when present in normal breast tissue; and

(c) classifying the test sample as: (i) normal breast tissue if the level of expression of the gene in the test stromal cell is not substantially higher than a control level of expression for a cell of the same type as the test stromal cell in normal breast tissue;
5 (ii) breast cancer tissue if the level of expression of the gene in the test stromal cell is substantially higher than a control level of expression for a cell of the same type as the test stromal cell in normal breast tissue.

10 11. The method of claim 10, wherein the stromal cells in the test sample and the standard samples are leukocytes and the genes are selected from those listed in Tables 7 and 15.

12. The method of claim 11, wherein the gene encodes interleukin-1 β (IL β) or
15 macrophage inhibitory protein 1 α (MIP1 α).

13. The method of claim 10, wherein the stromal cells in the test sample and the standard samples are myoepithelial cells or myofibroblasts and the genes are selected from those listed in Tables 8, 15, and 16.

20 14. The method of claim 13, wherein the gene encodes a polypeptide selected from the group consisting of cathepsins F, K, and L, MMP2, PRSS11, thrombospondin 2, SERPING1, cystatin C (CST3), TIMP3, platelet-derived growth factor receptor β -like (PDGFRBL), a collagen, collagen triple helix repeat containing 1 (CTHRC1), CXCL12, and
25 CXCL14.

15. The method of claim 10, wherein the stromal cells in the test sample and the standard samples are endothelial cells and the genes are selected from those listed in Tables 10 and 15.

16. The method of claim 10, wherein the stromal cells in the test sample and the standard samples are fibroblasts and the genes are selected from those listed in Table 15.

17. A method of diagnosis comprising:

5 (a) providing a test sample of breast tissue comprising a test stromal cell; and
(b) determining the level of expression in the stromal cell of a gene selected from those listed in Tables 7, 8, 10, and 15 wherein the gene is one that is expressed in a cell of the same type as the test stromal cell at a substantially higher level when present in normal breast tissue than when present in breast cancer tissue; and

10 (c) classifying the test sample as: (i) normal breast tissue if the level of expression of the gene in the test stromal cell is not substantially lower than a control level of expression for a cell of the same type as the test stromal cell in normal breast tissue;
(ii) breast cancer tissue if the level of expression of the gene in the test stromal cell is substantially lower than a control level of expression for a cell of the same type as the test
15 stromal cell in normal breast tissue.

18. The method of claim 17, wherein the stromal cells in the test sample and the standard samples are leukocytes and the genes are selected from those listed in Tables 7 and
20 15.

19. The method of claim 17, wherein the stromal cells in the test sample and the standard samples are myoepithelial cells or myofibroblasts and the genes are selected from those listed in Tables 8 and 15.

25 20. The method of claim 17, wherein the stromal cells in the test sample and the standard samples are endothelial cells and the genes are selected from those listed in Tables 10 and 15.

30 21. The method of claim 17, wherein the stromal cells in the test sample and the standard samples are fibroblasts and the genes are selected from those listed in Table 15.

22. A method of diagnosis comprising:

(a) providing a test sample of breast tissue comprising a test epithelial cell of the luminal epithelial type;

(b) determining the level of expression in the test epithelial cell of a gene selected from those listed in Tables 9 and 15, wherein the gene is one that is expressed in cancerous epithelial cells of the luminal epithelial cell type at a substantially higher level than those in normal breast tissue; and

(c) classifying the test sample as: (i) normal breast tissue if the level of expression of the gene in the test epithelial cell is not substantially higher than a control level of expression for an epithelial cell of luminal epithelial cell type in normal breast tissue; (ii) breast cancer tissue if the level of expression of the gene in the test epithelial cell is substantially higher than a control level of expression for an epithelial cell of the luminal epithelial type in normal breast tissue.

23. A method of diagnosis comprising:

(a) providing a test sample of breast tissue comprising a test epithelial cell of the luminal epithelial type; and

(b) determining the level of expression in the test epithelial cell of a gene selected from those listed in Tables 9 and 15, wherein the gene is one that is expressed in epithelial cells of the luminal epithelial cell type at a substantially lower level when present in breast cancer tissue than when present in normal breast tissue; and

(c) classifying the test sample as: (i) normal breast tissue if the level of expression of the gene in the test epithelial cell is not substantially lower than a control level of expression for an epithelial cell of luminal epithelial cell type in normal breast tissue; (ii) breast cancer tissue if the level of expression of the gene in the test epithelial cell is substantially lower than a control level of expression for an epithelial cell of the luminal epithelial type in normal breast tissue.

24. The method of claim 1, 2, 8, 9, 10, 17, 22, or 23, wherein the level of expression of the gene is determined as a function of the level of protein encoded by the gene.

25. The method of claim 1, 2, 8, 9, 10, 17, 22, or 23, wherein the level of expression of the gene is determined as a function of the level of mRNA transcribed from the gene.

26. A method of inhibiting proliferation or survival of a breast cancer cell, the method comprising contacting a breast cancer cell with a polypeptide that is encoded by a gene selected from those listed in Tables 1, 7-10, and 15, wherein the gene is expressed in the cancer cell, or a stromal cell in a tumor comprising the cancer cell, at a level substantially lower than in a normal cell of the same type.

27. The method of claim 26, wherein the cancer cell is in a mammal.

28. The method of claim 27, wherein the mammal is a human.

29. The method of claim 27, wherein the contacting comprises administering the polypeptide to the mammal.

30. The method of claim 27, wherein the contacting comprises administering a polynucleotide encoding the polypeptide to the mammal.

31. The method of claim 27, the method comprising:
(a) providing a recombinant cell that is the progeny of a cell obtained from the mammal and has been transfected or transformed *ex vivo* with a nucleic acid encoding the polypeptide; and

(b) administering the recombinant cell to the mammal, so that the recombinant cell expresses the polypeptide in the mammal.

32. A method of inhibiting pathogenesis of a breast cancer cell or stromal cell in a tumor of a mammal, the method comprising

(a) identifying a mammal with a breast cancer tumor; and

(b) administering to the mammal an agent that inhibits binding of a polypeptide encoded by a gene selected from those listed in Tables 2-10, 15, and 16 to its receptor or ligand,

wherein the gene is expressed in a breast cancer cell in the tumor, or in a stromal cell in the tumor, at a level substantially higher than in a corresponding cell in a non-cancerous breast, and

wherein the polypeptide is a secreted polypeptide or a cell-surface polypeptide.

33. The method of claim 32, wherein the agent is a non-agonist antibody that binds to the polypeptide.

34. The method of claim 32, wherein the agent is a soluble form of the receptor.

35. The method of claim 32, wherein the agent is a non-agonist antibody that binds to the receptor or ligand.

36. The method of claim 32, wherein the polypeptide is CXCL12.

37. The method of claim 32, wherein the receptor is CXCR4.

38. The method of claim 32, wherein the polypeptide is CXCL14.

39. The method of claim 32, wherein the receptor is a receptor for CXCL14.

40. A method of inhibiting expression of a gene in a cell, the method comprising introducing into a target cell selected from the group consisting of (a) a breast cancer cell and (b) stromal cell in a tumor comprising a breast cancer cell, an agent that inhibits expression of a gene selected from those listed in Tables 2-10, 15 and 16, wherein the gene is expressed in the target cell at a level substantially higher than in a corresponding cell in normal breast tissue.

41. The method of claim 40, wherein the agent is an antisense oligonucleotide that hybridizes to an mRNA transcribed from the gene.

42. The method of claim 41, wherein the introducing step comprises
5 administration of the antisense oligonucleotide to the target cell.

43. The method of claim 40, wherein the agent is a small molecule that inhibits expression of the gene.

44. The method of claim 41, wherein the introducing step comprises
10 administering to the target cell a nucleic acid comprising a transcriptional regulatory element (TRE) operably linked to a nucleotide sequence complementary to the antisense oligonucleotide, wherein transcription of the nucleotide sequence inside the target cell produces the antisense oligonucleotide.

45. The method of claim 40, wherein the agent is an RNAi molecule, and wherein one strand of the RNAi molecule hybridizes to a mRNA transcribed from the gene.

46. The method of claim 40, wherein the gene encodes CXCL12.

47. The method of claim 40, wherein the gene encodes CXCR4.

48. The method of claim 40, wherein the gene encodes CXCL14.

49. The method of claim 40, wherein the gene encodes a receptor for CXCL14.

50. A single stranded nucleic acid probe comprising:

(a) the nucleotide sequence of a tag selected from those listed in Tables 1-5, 7-
10, 15 and 16; or

(b) the complement of the nucleotide sequence.

51. An array comprising a substrate having at least 10 addresses, wherein each address has disposed thereon a capture probe comprising a nucleic acid sequence consisting of a tag nucleotide sequence selected from those listed in Tables 1-5, 7-10, 15, and 16.

5 52. The array of claim 51, wherein the tag nucleotide sequence corresponds to a gene encoding a protein selected from the group consisting of fatty acid synthase (FASN), trefoil factor 3 (TFF3), X-box binding protein 1 (XBP1), interferon alpha inducible protein 6-16 (IFI-6-16), cysteine-rich protein 1 (CRIP1), interferon-stimulated protein 15 kDa (ISG15), interferon alpha inducible protein 27 (IFI27), brain expressed X linked 1 (BEX1),
10 helicase/primase protein (LOC150678), anaphase promoting complex subunit 11 (ANAPC11), Fer-1-like 4 (FER1L4), psoriasin, connective tissue growth factor (CTGF), regulator of G-protein signaling 5 (RGS5), paternally expressed 10 (PEG10), osteonectin (SPARC), LOC51235, CD74, MGC23280, Invasive Breast Cancer 1 (IBC-1), Apolipoprotein D (APOD), carboxypeptidase B1 (CPB1), retinal binding protein 1 (RBP1),
15 FLJ30428, calmodulin-like skin protein (CLSP), nudix (NUDT8), MGC14480, interleukin-1 β (IL β), macrophage inhibitory protein 1 α (MIP1 α), cathepsins F, K, and L, MMP2, PRSS11, thrombospondin 2, SERPING1, cystatin C (CST3), TIMP3, platelet-derived growth factor receptor β -like (PDGFRBL), a collagen, collagen triple helix repeat containing 1 (CTHRC1), CXCL12, CXCL14, and a protein encoded by a gene identified by a SAGE tag
20 consisting of the nucleotide sequence CTGGGCGCCC.

53. The array of claim 51, wherein the array comprises at least 25 addresses.

54. The array of claim 51, wherein the array comprises at least 50 addresses.

25 55. The array of claim 51, wherein the array comprises at least 100 addresses.

56. The array of claim 51, wherein the array comprises at least 200 addresses.

30 57. The array of claim 51, wherein the array comprises at least 500 addresses.

58. A kit comprising at least 10 probes, each probe comprising a nucleic acid sequence comprising a tag nucleotide sequence selected from those listed in Tables 1-10, 15 and 16.

5 59. The kit of claim 58, wherein the kit comprises at least 25 probes.

60. The kit of claim 58, wherein the kit comprises at least 50 probes.

61. The kit of claim 58, wherein the kit comprises at least 100 probes.

10 62. The kit of claim 58, wherein the kit comprises at least 200 probes.

63. The kit of claim 58, wherein the kit comprises at least 500 probes.

15 64. A kit comprising at least 10 antibodies each of which is specific for a different protein encoded by a gene identified by a tag selected from the group consisting of the tags listed in Tables 1-5, 7-10, 15 and 16.

20 65. The kit of claim 64, wherein the antibodies are specific for a protein selected from the group consisting of fatty acid synthase (FASN), trefoil factor 3 (TFF3), X-box binding protein 1 (XBP1), interferon alpha inducible protein 6-16 (IFI-6-16), cysteine-rich protein 1 (CRIP1), interferon-stimulated protein 15 kDa (ISG15), interferon alpha inducible protein 27 (IFI27), brain expressed X linked 1 (BEX1), helicase/primase protein (LOC150678), anaphase promoting complex subunit 11 (ANAPC11), Fer-1-like 4
25 (FER1L4), psoriasin, connective tissue growth factor (CTGF), regulator of G-protein signaling 5 (RGS5), paternally expressed 10 (PEG10), osteonectin (SPARC), LOC51235, CD74, MGC23280, Invasive Breast Cancer 1 (IBC-1), Apolipoprotein D (APOD), carboxypeptidase B1 (CPB1), retinal binding protein 1 (RBP1), FLJ30428, calmodulin-like skin protein (CLSP), nudix (NUDT8), MGC14480, interleukin-1 β (IL β), macrophage
30 inhibitory protein 1 α (MIP1 α), cathepsins F, K, and L, MMP2, PRSS11, thrombospondin 2, SERPING1, cystatin C (CST3), TIMP3, platelet-derived growth factor receptor β -like

(PDGFRBL), a collagen, collagen triple helix repeat containing 1 (CTHRC1), CXCL12, CXCL14, and a protein encoded by a gene identified by a SAGE tag consisting of the nucleotide sequence CTGGGCGCCCC.

- 5 66. The kit of claim 64, wherein the kit comprises at least 25 antibodies.
67. The kit of claim 64, wherein the kit comprises at least 50 antibodies.
68. The kit of claim 64, wherein the kit comprises at least 100 antibodies.
- 10 69. The kit of claim 64, wherein the kit comprises at least 200 antibodies.
70. The kit of claim 64, wherein the kit comprises at least 500 antibodies.
- 15 71. A method of identifying the grade of a DCIS, the method comprising:
- (a) providing a test sample of DCIS tissue;
- (b) using the array of claim 51 to determine a test expression profile of the sample;
- (c) providing a plurality of reference profiles, each derived from a DCIS of a
- 20 defined grade, wherein the test expression profile and each reference profile has a plurality of values, each value representing the expression level of a gene corresponding to a tag selected from those listed in Tables 1-5, 7-10, 15, and 16; and
- (d) selecting the reference profile most similar to the test expression profile, to thereby identify the grade of the test DCIS.
- 25 72. A method of determining whether a breast cancer is a DCIS or an invasive breast cancer, the method comprising:
- (a) providing a test sample of breast cancer tissue;
- (b) determining the level of expression of CXCL14 in myofibroblasts in the
- 30 test sample;

(c) determining whether the level of expression of CXCL14 in the myofibroblasts in the test sample more closely resembles the level of expression of CXCL14 in control myofibroblasts of (i) DCIS or (ii) invasive breast cancer; and

(d) classifying the test sample as: (i) DCIS if the level of expression of CXCL14 in myofibroblasts in the test sample more closely resembles the level of expression of CXCL14 in control myofibroblasts of DCIS; (ii) invasive breast cancer if the level of expression of CXCL14 in myofibroblasts in the test sample more closely resembles the level of expression of CXCL14 in control myofibroblasts of invasive breast cancer.

73. An isolated DNA comprising:

- (a) the nucleotide sequence of a tag selected from those listed in Fig. 7; or
- (b) the complement of the nucleotide sequence.

74. A vector comprising the DNA of claim 73.

75. The vector of claim 74, wherein the DNA is operatively linked to a transcriptional regulatory element (TRE).

76. A cell comprising the vector of claim 74.

77. An isolated polypeptide encoded by the DNA of claim 73.